respond to the Office Action is requested, and the fee therefor accompanies this response.

Reconsideration of the application in light of the following remarks is respectfully requested.

REMARKS

The Present Invention:

The present invention relates to vaccine adjuvant compositions. The discovery at the core of this invention was that certain oil-in-water emulsions having oil droplets substantially all of which are less than 1 micron in diameter exhibit unexpectedly high adjuvant activity. This high activity means that these adjuvants can be used as the active adjuvant component of antigenic compositions for the treatment and/or prophylaxis of infection with pathogens. These adjuvant compositions represent a significant advance over the only adjuvant currently approved for human use ("alum", i.e., aluminum salts). As used herein, an adjuvant means a substance that increases the immune response to an antigen when administered with the antigen.

Office Action:

Applicants acknowledge with appreciation the withdrawal of the rejections under 35 U.S.C. § 112, first and second paragraphs, as well as the rejection under 35 U.S.C. § 102(b) and the removal of Mizushima, U.S. 4,613,505, as a reference.

Claims 1-9, 29, and 36 are currently pending in the application. These claims stand rejected under 35 U.S.C. § 103 as allegedly being obvious over Hoskinson, et al., U.S. 5,109,026 and Glass, U.S. 3,919,411, in view of Idson, Pharmaceutical Dosage Forms, Disperse Systems Vol. 1 (Lieberman, et al., eds.), Marcel Dekker, New York NY,1988, pp. 199-243 and Remington's Pharmaceutical Sciences (Gennaro, ed.), Mack Publ. Co., Easton PA, 1985, pp. 298-99, 317-29, and 1507-11.

This rejection is addressed below and, as explained, Applicants submit that this rejection should be withdrawn and that the claims are in condition for allowance.

Rejections Under 35 U.S.C. § 103:

Applicants respectfully submit that the combination of Glass and Hoskinson does not render obvious the present invention. Neither reference, either alone or in combination, teaches or

suggests submicron oil-in-water adjuvant compositions. Instead, both references relate to the use of an emulsion with another agent. The addition of the Remington and Idson references does not render obvious submicron oil-in-water adjuvants because these citations merely discuss known adjuvants (e.g., Idson, and Freund's adjuvants) and summarize known physical properties of emulsion formulations (Idson and Remington). There is no hint in this combination of references that submicron oil-in-water emulsions can themselves cause an adjuvant effect.

Hoskinson teaches that the combination of polycationic polyelectrolyte immunoadjuvants and oil emulsions is useful for stimulating an immune response against an antigen; it is the combination that is taught to be superior, not the oil emulsions themselves. There is no teaching or suggestion to enhance the activity of oil emulsion adjuvants without the addition of a second specified adjuvant -- in this case, a polycationic polyelectrolyte adjuvant.

Glass describes an "adjuvant" as a "substance that operates as a binder, carrier, or suspending vehicle for immunogens … the function of which is to increase the effectiveness of the agent or the immunogenic response from an immunogenic agent by virtue of the retardation and slowing down of the absorption of such

immunogens ... into the host's system ... " (col. 1, lines 46-54). This definition is for a depot-type adjuvant. The reference relates to the use of a macromolecular synthetic resin suspended in the aqueous phase of an emulsion, wherein an antigen binds to the resin. Glass's adjuvant system has very different properties from Applicants' adjuvant compositions. Specifically, Glass's adjuvants act through depot action (i.e., material holding the antigen at the injection site so that the antigen is slowly released).

Applicants' adjuvant compositions do not serve as depots for antigen release. Instead, the antigen, as well as the adjuvant itself, is rapidly dispersed from the site of introduction. It appears that the Office Action questions the sufficiency of Applicants' evidence on the rapid dispersion of the present adjuvant compositions from their site of injection. The Office Action notes (page 3), "It is respectfully noted that depot effects from Glass et al. and from Applicants are likely to be comparable." Applicants strongly disagree with this statement, and refer the reader to Ott et al., in Vaccine Design: The Subunit and Adjuvant Approach (ed. Powell et al.), Plenum Press, New York NY, 1995, pp. 277-96 (duplicate copy enclosed). On

pages 285-88, the mechanisms of adjuvant activity for the present compositions are analyzed experimentally.

In an experiment described therein, Applicants' adjuvant composition ("MF59") was radiolabeled as was a viral antigen, gD from Herpes Simplex Virus type 2 (HSV gD2), and these materials were given to rabbits as a standard IM injection. Upon examination of the muscle tissue at the injection site, at 6 hours post-injection, the oil from Applicants' adjuvant was only 10% of the original amount (this decreased to 5% over a 120 hour period). Likewise, the antigen also dispersed quickly: only 25% of the injected dose was found at the injection site after 6 hours (this decreased to < 0.05% after 120 hours). It is interesting to note that the antigen and adjuvant did not disperse at the same rates, again indicating no physical association. Both the antigen and the adjuvant quickly disperse from the injection site.

As further evidence of the independent effect of Applicants' adjuvants, the reader is referred to pages 286-87 of the same chapter, wherein the independent activities of the antigen and adjuvant were demonstrated in vivo by administration of the antigen and adjuvant into proximate sites at different times. As can be seen from Figure 8 (page 287), administration of the

adjuvant at times ranging from 24 hours before antigen injection to 1 hour after antigen injection all resulted in high antibody titers. Administration of the adjuvant 24 hours post-injection, however, resulted in reduced antibody titers. Again, these results support the conclusion that Applicants' submicron oil-in-water adjuvant compositions activate the immune system independently of the antigen, and that such activation persists for at least 24 hours. Giving the adjuvant after the antigen has dispersed from the site results in decreased antibody titers because the adjuvant effect of the present oil-in-water compositions cannot be utilized. In addition, antigens that are fully adjuvanted by Applicants' compositions have been shown to have no measurable binding to the submicron oil droplets (e.g., experimental data on HSV gD discussed on page 286 of the above-referenced article). All of this data contradicts the suggestion that Applicants' compositions work through some sort of depot effect wherein an emulsion would hold an antigen at the injection site.

Finally, Applicants submit the enclosed Declaration under 37 C.F.R. § 1.132, wherein it is demonstrated experimentally that not only do the antigen and adjuvant need not be administered simultaneously, they also do not need to be administered at the

same site! In the described experiment, one group of rabbits was injected only with an antigen (HSV gD2) whereas the second group was injected with the antigen in one thigh and the adjuvant ("MF59") in the opposite thigh. Again, Applicants' adjuvant compositions stimulated antibody titers three-fold higher after one immunization, and fifteen-fold higher after the second immunization. The adjuvant's effect is independent of the site of antigen administration; again demonstrating that there is no antigen depot effect such as that taught by Glass. Applicants submit that this evidence completely rebuts the suggestion made in the present Office Action that "depot effects from Glass et al. and from Applicants are likely to be comparable." Under 37 C.F.R. § 1.107(b), Applicants hereby request that, if the Examiner is basing this assertion on facts within his personal knowledge, then such knowledge must be supported by an affidavit.

Furthermore, there is absolutely no teaching in Hoskinson or Glass that such oil emulsion combinations should be submicron in size. The addition of Idson and Remington to these references does not render obvious the present invention. Both of these standard formulation texts refer to pharmaceutical emulsions, wherein small particle size increases stability of the emulsion. Only on page 224 of Idson is any mention of adjuvants; and again

reference is made only to depot effects ("slow release") of water-in-oil Freund's-like adjuvants. Remington does not add anything further to the description of such emulsions found in Idson, but rather summarizes physical properties of such emulsions.

As previously mentioned, Applicants were the first to discover that submicron oil-in-water emulsions could themselves function as adjuvant compositions, with or without the presence of additional adjuvants. These adjuvants do not need to be administered physically or simultaneously with the antigen for an immunostimulatory effect to be seen. Furthermore, these adjuvants are not functioning through a depot effect, as shown by the referenced experimental work Applicants respectfully request that the rejection under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

In view of the remarks contained herein, Applicants respectfully request that the current rejections be withdrawn and that Claims 1-9, 29, and 36 be allowed.

If the Examiner believes that a telephone interview would expedite prosecution of this application, the Examiner is invited to contact the undersigned at (510) 923-2708.

Respectfully submitted,

Dated: May 14, 1997

By: Barbara G. McClung
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Chapter 10

MF59

Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines

Gary Ott, Gail L. Barchfeld, David Chernoff, Ramachandran Radhakrishnan, Peter van Hoogevest, and Gary Van Nest

1. RATIONALE FOR AND DESIGN OF MICROFLUIDIZED OIL/WATER EMULSIONS

Advances in recombinant DNA technology have made possible the advent of a new generation of safer, better-defined subunit vaccines. Because vaccines based on these weakly immunogenic antigens require an adjuvant for efficacy, we undertook the development of a safe and efficacious adjuvant suitable for widespread human administration. Vaccines formulated with aluminum salts (alum), the only adjuvant thus far utilized with vaccines approved in the United States for human administration, were necessarily adopted as a benchmark for the minimum acceptable activity of a new adjuvant. Our goal was to develop an adjuvant that significantly exceeded aluminum hydroxide in potency, while retaining equally low toxicity. By the early 1990s a wide variety of approaches to adjuvant development had been described (Allison and Byars, 1990; Edelman, 1980; Gregoriadis and Panagiotidi, 1989; Warren *et al.*, 1986). Two major mechanisms of adjuvant activity have been repeatedly cited in this literature: the depot effect, whereby long-term release of antigen results in increased immune response; and coadministration of immunostimulators, which specifically activate portions of the immune system in, as yet, incompletely defined fashions. The prototypic strong adjuvant, complete Freund's adjuvant (CFA),

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Vaccine Design: The Subunit and Adjuvant Approach, edited by Michael F. Powell and Mark J. Newman. Plenum Press, New York, 1995.

combined these functions by releasing a mixture of immunostimulatory mycobacteri wall components along with antigen from a water/mineral oil/Arlacel A emulsion over an extended period of time (Freund, 1956). CFA remains the reference standa potent adjuvant activity; however, it is now considered too toxic in many cases fc even in laboratory animals. In addition to the aluminum salts, several adjuvants bas the depot effect alone have been studied. These include incomplete Freund's adj (IFA), which lacks the potent, but toxic, cell wall components, and Adjuvant 65 (wate nut oil/mannide monooleate), a yet further detoxified water/oil formulation (Hillem *al.*, 1972a,b). Despite extensive study, neither formulation was approved for h administration. We chose to avoid water/oil emulsions. A more recent version of the approach, controlled release of antigen from synthetic polymer microspheres, rema promising area of study (Cohen *et al.*, 1991; O'Hagan *et al.*, 1991) but appears to ha unacceptably long development time for our purposes.

A major part of the long ongoing effort to develop immunostimulators as adju has been devoted to characterization and synthesis of mycobacterial cell wall cor nents and their analogues (White *et al.*, 1964). The most studied component of the wall, the muramyl peptide *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP), synthesized by 1975 (Kotani *et al.*, 1975; Merser *et al.*, 1975) and the activit a number of analogues has since been described (Ott *et al.*, 1992). We initiall out to develop an adjuvant that used the amphiphilic muramyl tripeptide, MTF [sodium *N*-acetyl-muramyl-L-alanyl-D-isoglutaminyl-L-alanyl-2-(1′,2′-dipa toyl-*sn*-glycero-3′-phospho)ethylamide] (Gisler *et al.*, 1986). MTP-PE was lected because of an established clinical record of high potency and low toxi (Fidler, 1988; Fidler *et al.*, 1981) as well as the availability of sufficient quantiti injectable-grade material.

Because muramyl peptides alone have been reported to be no more effectiv adjuvant than alum (Audibert *et al.*, 1980) they have been formulated with a variet vehicles. The use of liposomes containing muramyl peptides has been described for se systems (Brynestad *et al.*, 1990; Gregoriadis and Manesis, 1980; Ullrich and Fidler, 19 It has been our experience that more robust antibody responses are obtained with oil/w emulsions (Sanchez-Pescador *et al.*, 1988). Complex squalene/water emulsions contai a muramyl peptide component, trehalose dimycolates, and monophosphoryl lipid A h been shown to be effective adjuvants (Masihi *et al.*, 1986; Ribi *et al.*, 1976). A bod work has been devoted to generation of less complex and better defined variations of t formulations. Similar emulsions have been used to formulate a variety of hydroph spreading agents (Woodward, 1989). A family of potent synthetic hydrophobic agents, pluronic block polymers, have been used with both squalene emulsions (Hunter Bennett, 1984; Hunter *et al.*, 1981) and squalane emulsions in combination with synthetic muramyl peptide, threonyl MDP (Byars and Allison, 1987; Byars, *et al.*, 19 Finally, the hydrophobic muramyl peptide B30-MDP has also been formulated wi Ribi-like emulsion (Tsujimoto *et al.*, 1986).

Our initial formulations were designed with the intention of binding MTP-PE to surface of a squalene/water emulsion. The formulation MTP-LO, a prototype formula generated by multiple intersyringe passages, had excellent adjuvant activity in guinea p (Sanchez-Pescador *et al.*, 1988). Poor physical stability and the modest adjuvant acti

observed in preclinical studies with large animals and clinical trials with the HIV coat protein antigen env2-3 (Wintsch *et al.*, 1991) led to a series of efforts to improve the emulsion by emulsification under higher shear forces and to modification of surfactant composition with the intent of generating a stable, sterile-filterable formulation that would be compatible with a variety of antigens and buffer systems. Higher shear forces were first applied to the MTP-LO formulation by passage through a Kirkland "knife-edge valve" homogenizer (Kirkland Instruments, El Cajon, CA). The emulsion, K-LO, had a mean droplet diameter of ~700 nm, but suffered from flocculation (creaming) after storage for 2 h. Homogenization of the same formulation with a Microfluidizer® (Microfluidics, Newton, MA) resulted in an emulsion, MF-LO, with a droplet diameter of ~ 400 nm. However, this emulsion coalesced on exposure to PBS (0.15 M NaCl, 0.01 M sodium phosphate). While higher energy homogenization produced improved emulsions, the original concentrations of Tween 80 and MTP-PE were not sufficient to obtain an oil/water emulsion with the desired characteristics.

In order to investigate the emulsifying potential of MTP-PE alone, a series of MTP-PE/squalene emulsions were prepared using the Microfluidizer®. Emulsion diameters were determined in water and in PBS after a 2-h incubation (Fig. 1). While stable MTP-PE/squalene emulsions of nearly constant diameter could be generated in water, the diameter in the presence of PBS was strongly dependent on the MTP-PE concentration. The interdependence of potentially critical parameters (squalene dose, MTP-PE dose, and droplet diameter) was a potential source of difficulty with these formulations. A single example, MF1, was employed for preclinical testing.

Stabilization with Tween 80, which had been used as a minor component in the squalene formulations previously described (Byars and Allison, 1987; Hunter *et al.*, 1981; Ribi *et al.*, 1976), was an attractive alternative to using MTP-PE as the emulsifier. In addition, emulsions stabilized with mixtures of Tween 80 and the homologous low HLB spreading agent Span 85 were studied. These emulsions offered the possibility of both greater long-term stability (Boyd *et al.*, 1972) and the potential for an adjuvant contribution from the spreading agent (Woodward, 1989). Figure 2 shows the mean droplet diameter

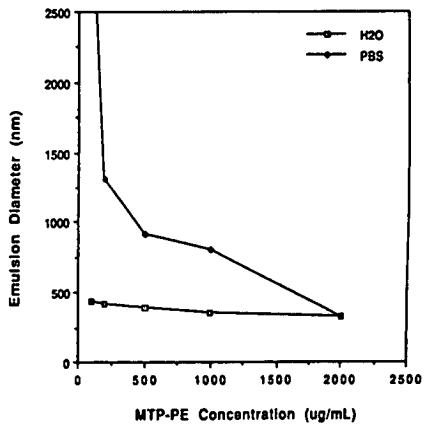

Figure 1. Dependence of emulsion diameter on MTP-PE concentration. 4.3% (w/v) squalene was emulsified with MTP-PE at 12,000 psi (Microfluidizer®) in water and emulsion diameters were determined by photocorrelation spectroscopy on samples in water or phosphate-buffered saline.

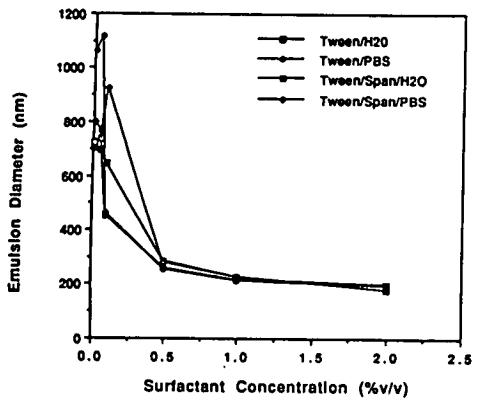

Figure 2. Dependence of emulsion diameter on total surfactant concentration. 4.3% (w/v) squalene was emulsified with either Tween 80 or a 50:50 mixture of Tween 80 and Span 85 at 12,000 psi (Microfluidizer®) in water and emulsion diameters were determined in water or phosphate-buffered saline.

obtained by microfluidization of a series of 4.3% (w/v) squalene emulsions stabilized with varying inputs of either Tween 80 or a 50:50 mixture of Tween 80 and Span 85. At total detergent concentrations greater than 0.5% (w/v), emulsions of <300 nm diameter were produced with either surfactant composition. The droplet diameter of these emulsions was not sensitive to incubation in the presence of physiological saline, and they showed no sign of either creaming or coalescence over a period of months. In addition, we were able to sterilize these emulsions by passage through a 0.22-μm filter. These properties led us to focus development on two of these formulations: MF59 (4.3% w/v squalene, 0.5% w/v Tween 80, 0.5% w/v Span 85) and MF69 (4.3% w/v squalene, 0.25% w/v Tween 80, 0.75% w/v Span 85). Homogenization of a modified MF59 formulation containing 100 μg/dose MTP-PE (MF59-100) resulted in an emulsion that was indistinguishable in size from the MF59-0 vehicle. A series of MTP-PE-containing formulations have been used and the MTP-PE dose is designated in hyphenated style. The MF59 emulsion system thus fulfilled our requirement for a vehicle where size and stability were independent of the presence of MTP-PE. Determination of the degree of interaction between the emulsion and MTP-PE was carried out by separating the emulsion droplets from the aqueous phase by flotation in a discontinuous sucrose gradient. MTP-PE concentration was determined both for the upper fraction where the emulsion had been concentrated and for the lower fractions where free MTP-PE remained. Figure 3 shows the distribution of MTP-PE in the gradient for microfluidized MF59-100 (Fig. 3A) and for a control containing 100 μg of MTP-PE alone (Fig. 3B). Approximately 50% of the MTP-PE is found in the uppermost fraction with the emulsified squalene droplets. This result was compared with the distribution achieved after incubation of 100 μg MTP-PE with MF59-0 (data not shown). After 48 h incubation, ~50% binding was observed. Incubation of the MTP-PE/MF59 complex isolated from the uppermost gradient fraction with PBS for 72 h resulted in dissociation of less than 10% of the bound MTP-PE. These in vitro experiments indicated that MF59 could function as an MTP-PE carrier and would be suitable for preclinical testing.

In summary, preliminary formulation work on squalene/H_2O emulsions provided three candidates for second-generation preclinical testing in a variety of species: (1)

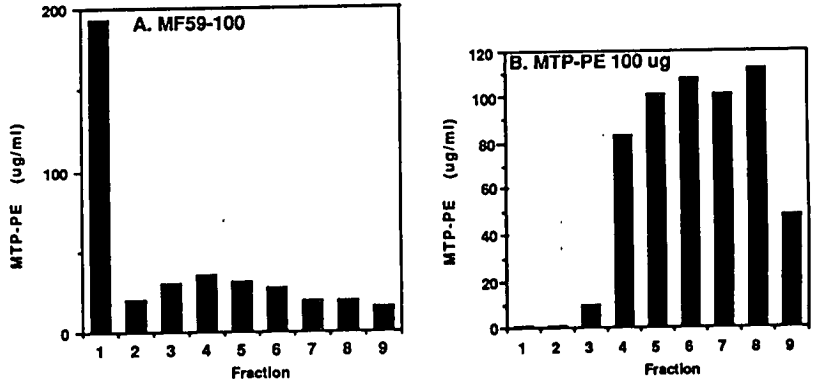

Figure 3. Sucrose gradient distribution of emulsion-bound and free MTP-PE. Either MF59-100 or 100 μg MTP-PE in 55% (w/v) sucrose was overlaid with phosphate-buffered saline and centrifuged for 60 min at 20,000 rpm in an SW55 Ti. Nine 0.5-mL fractions were collected from the top of the centrifuge tube and MTP-PE was determined by RP-HPLC.

well-homogenized versions of the parent MTP-LO formulation, (2) the rather closely related MTP-PE-stabilized MF-1 formulation, and (3) the nonionically stabilized MF59/69 emulsions, which can serve as carriers for MTP-PE but do not require it for stability.

2. PRECLINICAL EXPERIENCE WITH MF59

While testing the early low-oil-emulsion formulations, it became apparent that significant differences exist among animal species with respect to the ability of the animals to respond to particular adjuvant formulations. This is illustrated in Fig. 4, which shows the anti-gD2 responses of guinea pigs and baboons that received different adjuvant formulations. In guinea pigs, gD2 adsorbed to aluminum hydroxide produced low antibody titers (mean titer 140) compared to MTP-PE/LO (mean titer 5200), the insufficiently stable prototype emulsion formulation. The stable formulation MF59 produced titers equivalent to MTP-PE/LO (mean titer 4800). In baboons, however, both MTP-PE/LO and the MTP-PE-stabilized emulsion MF1 generated antibody titers (mean titers respectively 7385 and 7800) similar to aluminum hydroxide (4900). In contrast, immunization with MF59 resulted in fivefold higher titers (mean titer 26000) than the other three adjuvants (Van Nest *et al.*, 1992). We have consistently observed this species-dependent difference in response to low-oil-emulsion adjuvants. Mice, guinea pigs, and rabbits respond well to most of the low-oil formulations, regardless of the emulsion droplet size or stability. Goats, baboons, and chimpanzees require a stable, small-droplet-diameter emulsion for optimal responses. This requirement has also proven to be the case in human clinical trials. The particular antigen used does not alter this effect. It is not clear whether there is a simple physiological basis for this difference between small and large animals, or if perhaps there

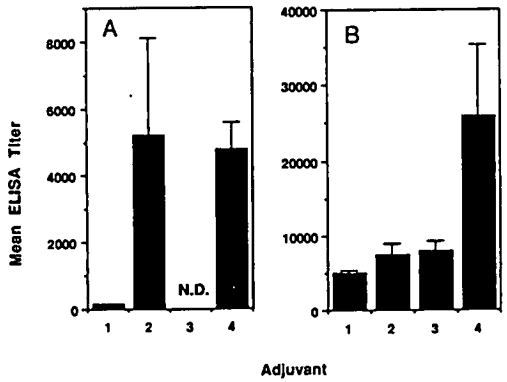

Figure 4. Performance of different adjuvant formulations with HSV gD2 in guinea pigs and baboons. Animals were immunized three times at 3-week intervals with recombinant gD2 produced in Chinese hamster ovary (CHO) cells combined with different adjuvant formulations. Adjuvants: (1) aluminum hydroxide (Alhydrogel); (2) MTP-PE-LO; (3) MF1; (4) MF59. gD2 doses used were 12.5 μg for guinea pigs and 25 μg for baboons. Aluminum hydroxide doses were 42 μg for guinea pigs and 85 μg for baboons. With MTP-PE-LO, MF1, and MF59 formulations, 50-μg doses of MTP-PE were used for guinea pigs and 100 μg for baboons. Two weeks after the third immunization, animals were bled, and anti-gD2 antibody titers were determined by ELISA. ELISA values represent the geometric mean and standard error of groups of five animals. (A) Guinea pig titers; (B) baboon titers. MF1 was not tested in guinea pigs. Reproduced by permission of Cold Spring Harbor Laboratory Press.

is an immunological basis for the differences. The pragmatic conclusion is that large animal models are more relevant for predicting adjuvant performance in humans.

MF59 has been extensively tested in a number of animal species with both recombinant and natural antigens. The animal models used include mice, guinea pigs, rabbits, goats, and several nonhuman primates including chimpanzees. The vaccine antigens tested include recombinant proteins and glycoproteins from HSV, HIV, HCV, CMV, HBV, HPV, and malaria as well as natural glycoproteins from the influenza virus. The specific animal and antigen combinations that have been studied are summarized in Table I. In all instances, the antigen/MF59 combinations generated high antigen-specific antibody titers and, where tested, high virus neutralizing titers. In cases where MF59 vaccines were compared with aluminum hydroxide-adsorbed antigen at equal antigen doses the MF59 titers were generally 3- to 50-fold higher than the alum titers. Several examples of the MF59 to alum comparisons are shown in Table II. In these examples, MF59 generated antibody titers 4- to 34-fold higher than alum, depending on both the antigen and the animal species studied. The potent CFA/IFA adjuvants have served as standards for effective adjuvants. When MF59 or other microfluidized low-oil emulsions that we have prepared were compared to CFA/IFA, different relative effects were seen with different animals and antigens. For instance, in rabbits immunized with HSV gD2 and CFA/IFA, titers at 2 weeks after the third immunization were 31,900 ± 1700, but for animals immunized with emulsion and MTP-PE titers were slightly higher at 50,500 ± 14,700. Conversely, *Aotus* monkeys

Table I
Experience with MF59 in Animal Models

Species	HSV	HIV	Influenza	HCV	CMV	HBV	HPV	Malaria
Mouse	+	+	+		+		+	+
Guinea pig	+	+	+		+			
Rabbit	+	+	+			+	+	
Goat	+	+	+			+		+
Aotus monkey								+
Rhesus macaque		+						
Baboon	+	+						
Chimp		+		+				

immunized with malaria SERA 1 antigen and either CFA/IFA or MF59 had titers of 692,000 ± 140,700 and 25,500 ± 11,200, respectively, 4 weeks after the third immunization (Inselberg *et al.*, 1993). MF59 gave higher antibody titers than IFA against gp120 when compared in baboons (Van Nest *et al.*, 1992). Overall, MF59 and related microfluidized low-oil emulsions generate antibody titers consistently higher than those obtained with aluminum hydroxide, equal to or higher than IFA, and equal to or lower than CFA/IFA.

The low-oil emulsions could function as MTP-PE carriers and MTP-PE was originally included as an immunostimulant in many MF59 formulations. In rabbits and guinea pigs with HSV and HIV antigens, MTP-PE has a demonstrable effect on specific antibody levels. A typical experiment is shown in Fig. 5 in which rabbits were immunized with HSV gD2 and MF59 containing from 0 to 1000 μg MTP-PE. At 2 weeks after the second immunization there is little difference in titers for animals immunized with 0 to 25 μg MTP-PE, but a marked increase is observed with 100 and 1000 μg of MTP-PE. After the third immunization, all MTP-PE doses show antibody titers higher than MF59-0, with the

Table II
Comparison of Alum and MF59 with Different Antigens in Several Animal Species[a]

Species	Antigen	ELISA titer with alum	ELISA titer with MF59	Ratio MF59:alum
Guinea pig[b]	HSV gD2	140 ± 30	4800 ± 400	34
Goat[b]	HSV gD2	170 ± 30	1600 ± 490	9
Goat[b]	HBVsAg	1500 ± 800	5300 ± 1900	4
Baboon[b]	HSV gD2	4900 ± 360	26,000 ± 9000	5
Baboon[c]	HIV gp120	1070 ± 590	28,300 ± 10,800	26

[a]Groups of five animals (except the HSV gD2–baboon experiment where groups of three were used) were immunized three times with the stated antigen and either alum or MF59 as adjuvant. Two weeks after the third immunization, the animals were bled and the antibody titers against the specific antigens were determined by ELISA.

[b]Experiments used MF59/MTP-PE (50 μg MTP-PE for guinea pigs, 100 for goats and baboons).

[c]Experiment used MF59-0.

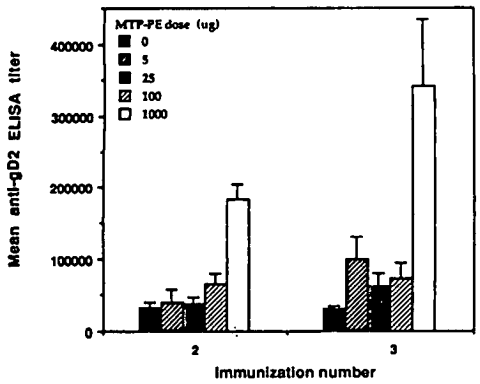

Figure 5. Effect of MTP-PE in the MF59 formulation on anti-gD2 antibody responses in rabbits. Groups of five animals were immunized three times at 3-week intervals with 25 μg gD2 and MF59 containing 0, 5, 25, 100, or 1000 μg MTP-PE. Two weeks after the second and third immunizations, animals were bled and anti-gD2 antibody titers were determined as in Fig. 4.

1000-μg dose clearly higher than the others. The MTP-PE dose effect was not found in experiments with larger animals. Table III shows the antibody responses of goats immunized three times at 4-week intervals with HSV gD2 and MF59, containing 10 to 1000 μg MTP-PE. Variability within the groups, indicated by standard errors, is greater than differences between the groups. MTP-PE provides no obvious benefit in this model. There is a similar absence of MTP-PE effect in baboons with both HSV and HIV antigens. Clinical studies (see Section 5) have confirmed the goat and baboon results and show no enhancement of antibody responses in humans with the inclusion of MTP-PE in the formulation. The immune-stimulating activities of MF59 are a property of the microfluidized emulsion and do not necessarily require the addition of other immunostimulatory factors.

The discovery that the emulsion was the primary adjuvant made squalene dose ranging studies advantageous. Preliminary preclinical studies utilized the prototypic emulsion (43 mg/mL squalene) in a 1:1 dilution with the antigen/PBS at the maximum volume appropriate for each species. Initial dose ranging consisted of varying squalene concentration around the prototypic value. Data from Fig. 6 show antibody titers obtained from guinea pigs vaccinated twice at a 3-week interval with 0.2 mL of MF59/influenza vaccine at doses

Table III

Effect of MTP-PE on the Antibody Response Induced with MF59 and gD2 in Goats[a]

MTP-PE dose (μg)	Anti-gD2 ELISA titer after 2 immunizations	Anti-gD2 antibody titer after 3 immunizations
10	1318 ± 702	899 ± 253
100	717 ± 416	435 ± 286
500	960 ± 526	471 ± 204
1000	1194 ± 992	965 ± 700

[a]Groups of five animals were immunized three times at 4-week intervals with 25 μg gD2 and MF59 containing 10, 100, 500, or 1000 μg MTP-PE. Two weeks after the second and third immunizations, animals were bled and anti-gD2 antibody titers were determined by ELISA.

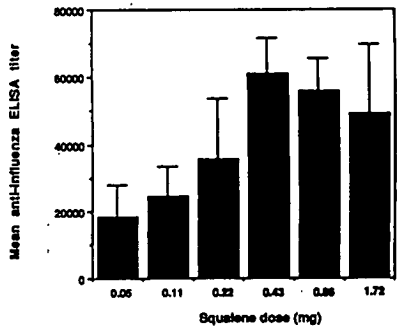

Figure 6. Effect of emulsion dose on antibody response to influenza vaccine in guinea pigs. Groups of five animals were immunized two times with a 3-week interval with 9 μg of trivalent influenza vaccine and MF59 containing 0.05, 0.11, 0.22, 0.43, 0.86, or 1.72 mg squalene. Two weeks after the second immunization the animals were bled and anti-influenza ELISA titers were determined as in Fig. 4.

ranging from 0.05 to 1.72 mg squalene. Antibody response increased with increasing doses up to 0.43 mg (mean titer 61,100) while larger doses of MF59 did not produce significantly higher titers (1.72 mg squalene produced a mean titer of 49,200). A similar plateau in the dose response was observed in goats immunized with 0.5 mL influenza/MF59-100 vaccine with doses ranging from 10.8 to 43 mg squalene. In this case, the prototypic dose of 10.8 mg squalene performed as well as the higher doses and was adopted for further studies.

A variety of toxicology tests have been done with both MF59 alone and in combination with HSV and HIV antigens. These studies have included both short-term protocols (2–3 immunizations at weekly or biweekly intervals or 14 daily immunizations) and long-term protocols (12 immunizations over 8 months). All studies were done using anticipated human doses and injection volumes in rabbits or dogs. The results of the studies indicate that while some changes in clinical laboratory parameters and histopathology were detected, all of the changes were minor and transient. Teratology studies in rats and rabbits indicated no embryotoxic or teratogenic effects of MF59 combined with HIV gp120. The overall conclusion from these studies is that the MF59 adjuvant formulation presents no safety problems either alone or combined with the antigens tested. The favorable toxicology profile of MF59 has allowed extensive clinical testing of the adjuvant in the general adult population and also in infants and children.

3. MECHANISM OF ADJUVANT ACTIVITY

To have rational adjuvant design, it is important to define the mechanism(s) of action for adjuvant systems that have significant efficacy. We have attempted to determine which features of MF59 contribute to its adjuvant activity. While a significant part of the activity of water/oil emulsions has been attributed to the long-term residence of antigen and adjuvant at the injection site (Freund, 1956), the depot effect does not appear to be significant for oil/water emulsions such as MF59. In a preliminary experiment to determine the rate of clearance of both MF59 emulsion and the HSV gD2 antigen from the injection site, either radiolabeled MF59 (^{125}I-iodinated squalene) or ^{125}I-HSV gD2 was administered to rabbits as a standard intramuscular injection. The muscle at the injection site was excised at times ranging from 6 to 120 h and radioactivity remaining in the tissue was determined. At 6 h, iodinated squalene at the injection site was only 10% of the input and decreased to

5% of the input over the 120-h period. For the antigen, 25% of the injected dose was found at the injection site after 6 h and this decreased to <0.05% at 120 h.

Despite our initial thought that MF59 could function as a carrier to transport MTP-PE to local lymph nodes, the data derived from large animal immunogenicity studies indicate a rather weak dependence of antibody titer on the dose of MTP-PE administered. While we have no data on actual delivery of MTP-PE to any critical site, substantial adjuvant effect is observed in the absence of any muramyl peptide; therefore, the emulsion vehicle is itself the source of adjuvant activity.

A potential basis for the adjuvant activity of MF59 was binding of antigen to the emulsion. The emulsion could be envisioned as a core for the formation of a pseudovirion particle. Such a particle might, like inactivated viruses, have increased immunogenicity derived from either facilitated transport to lymphoid tissues, or the effects of polyvalent arrayed antigen presentation. Adjuvant activity of synthetic particles has previously been discussed in terms of antigen binding (Kossovsky *et al.*, 1991; Kreuter *et al.*, 1986, 1988), and the SAF oil/water emulsion has been reported to bind antigen to the droplet surface (Allison and Byars, 1987). The interaction of the HSV antigens gD2 and gB2 with the MF59 emulsion was investigated by utilizing the sucrose gradient centrifugation method previously described for MTP-PE binding. After separation of the emulsion droplets from the original aqueous phase, no gD2 was found to be associated with the oil droplets. Since MF59 is effective at generating high-titer antibody to gD2, detectable binding of antigen to MF59 is not necessary for adjuvant activity. Incubation of HSV gB2 with MF59 for 48 h at 37°C resulted in ~10% binding and the MF59/gB2 complex was stable enough to be isolated by sucrose gradient centrifugation. Antibody titers generated by either free or bound gB2 (Fig. 7) indicate that at our typical antigen/emulsion ratio the effect of binding gB2 to MF59 is not significant. However, when the MF59 dose was reduced to 1% of the normal dose and gB2 was held constant, the titer obtained with bound protein was ~fourfold greater than that obtained with free gB2. Thus, at very low emulsion doses in which surface density of antigen is much greater, the effect of bound antigen becomes significant compared to the effect of emulsion alone, which dominates at higher MF59 doses. In order to further demonstrate the independent activities of antigen and adjuvant in vivo, the antigen and adjuvant portions of the vaccine were injected into proximate sites at different

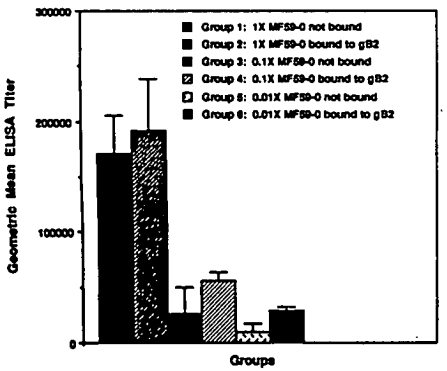

Figure 7. Effect on immunogenicity of binding HSV gB2 to MF59-0. Groups of eight animals were immunized three times at 3-week intervals with 4 μg gB2 and 4.3, 0.43, or 0.043 mg MF59-0. Protein was either bound to the emulsion by 48 h incubation or mixed with emulsion immediately prior to injection. Animals were bled 2 weeks after the third immunization and anti-gB2 titers determined by ELISA.

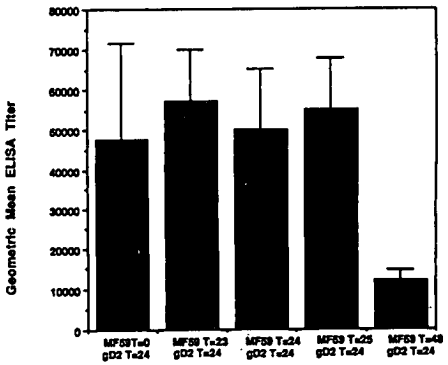

Figure 8. Effect on immunogenicity of temporally separated delivery of antigen and adjuvant. Five groups of eight rabbits were vaccinated with 25 μg HSV gD2 and 4.3 mg MF59-0 three times at 3-week intervals. Antigen and adjuvant were injected at proximal sites at times indicated. Animals were bled 2 weeks after the third immunization and anti-gD2 titers determined by ELISA.

times (Fig. 8). Administration of MF59 at times ranging from 24 h before antigen injection to 1 h after antigen injection resulted in indistinguishable antibody titers. Administration of MF59 24 h postinjection of gD2 resulted in a much reduced antibody titer. These results are consistent with the interpretation that MF59 droplets activate the immune system in the absence of antigen and that the activation persists for at least 24 h. Perhaps macrophage uptake of the emulsion droplets results in cytokine production, which leads to enhanced activity in the presence of antigen.

One clear feature in the action of MF59 vaccines is the ability of combined emulsion and antigen to stimulate production of a variety of cytokines. In one example of the cytokine responses to MF59, mice were immunized three times at weekly intervals with influenza vaccine alone or influenza vaccine combined with MF59-0. Three hours after the last immunization, animals were sacrificed. Draining lymph nodes were dissected and lymph node cell preparations were plated in culture medium without further stimulation. Twenty-four hours later, the culture media were collected and assayed for IL-2, IL-4, IL-5, and IL-6 levels by ELISA. As shown in Table IV, the vaccine alone did not induce production of detectable levels of IL-2 or IL-4 and induced only low levels of IL-5 and IL-6 (140 and 120 pg/ml, respectively). In contrast, vaccine plus MF59 induced much higher levels of

Table IV
Cytokine Response[a] at the Local Lymph Node after Injection of Mice with Influenza Vaccine Alone or Combined with MF59-0[b]

Immunogen	IL-2	IL-4	IL-5	IL-6
Influenza vaccine	<5	<5	140	120
Influenza vaccine + MF59	580	122	4050	825

[a]Cytokine responses are expressed as pg/mL in lymph node cell culture media.

[b]BALB/c mice were immunized subcutaneously three times at weekly intervals either with 9 μg of trivalent influenza vaccine alone or with the vaccine combined with MF59-0. Three hours after the third immunization, mice were sacrificed, draining lymph nodes were dissected, and lymph node cell suspensions were prepared. The lymph node cells were cultured 24 h without further restimulation and the media were assayed for IL-2, IL-4, IL-5, and IL-6 by ELISA.

all four cytokines. This stimulation was caused by the antigen and emulsion in the absence of any of the immunostimulatory molecules thus far associated with cytokine induction. The observation that an MF59-adjuvanted vaccine induces an elevated cytokine response is consistent with T-cell proliferation data obtained in clinical trials (R.L. Burke, personal communication). In this study, peripheral blood mononuclear cells were isolated at various times from individuals who were either naive with respect to HSV gD2, or had been vaccinated with gD2/alum or gD2/MF59. The frequency of gD2-responsive T cells was determined by a modified limiting dilution assay. Vaccination with MF59/gD2 resulted in 4- to 8-fold greater T-cell frequencies after two immunizations than those achieved with four vaccinations using gD2 adsorbed to aluminum hydroxide. In contrast to results obtained with alum, the MF59-induced gD2-dependent T-cell frequencies remained high during the 5-month interval between the second and third immunizations and were elevated to 6- to 15-fold over alum after that immunization.

While some data have been obtained concerning the mechanism of action of MF59 and related adjuvants, much more information is clearly needed. Up to this point, the primary efforts have been devoted to demonstration of clinical efficacy, which we hope will justify additional probes into the immunological mechanism of action.

4. MANUFACTURING AND SCALEUP OF MF59

The development of reliable high-pressure homogenization conditions was necessary to reproducibly manufacture large-scale submicron adjuvant emulsions for commercial use. Because no commercial instrument is suitable for the introduction of separate oil and aqueous phases, preemulsions were generated by mixing an oil phase, consisting of squalene and Span 85, with an aqueous solution of Tween 80 and homogenizing with a high-speed blade homogenizer. These preemulsions, which had droplet diameters in the range of 1 to 10 μm, were homogenized with a Microfluidizer® 110Y (Microfluidics, Newton, MA) to give the final submicron emulsions. Initially, 100- to 300-mL batches of emulsion were generated using five passes through the Microfluidizer® in a recycling mode. Droplet size distributions were determined by photocorrelation spectroscopy using a Coulter N4MD laser particle sizer. Droplet diameter data (pre and post sterile filtration)

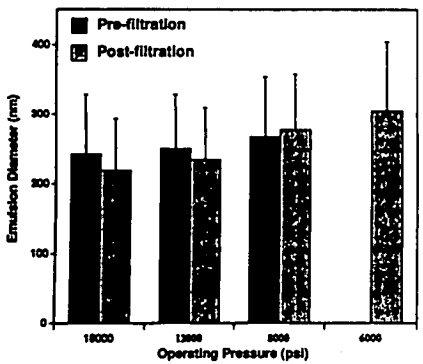

Figure 9. Effect of operating pressure on adjuvant emulsion droplet diameter. Emulsions were prepared by homogenization in a Microfluidizer® Model 110Y and were assayed for droplet diameter by photocorrelation spectroscopy using a Coulter N4MD submicron particle analyzer. Error bars indicate average standard deviation around the unimodal mean diameter.

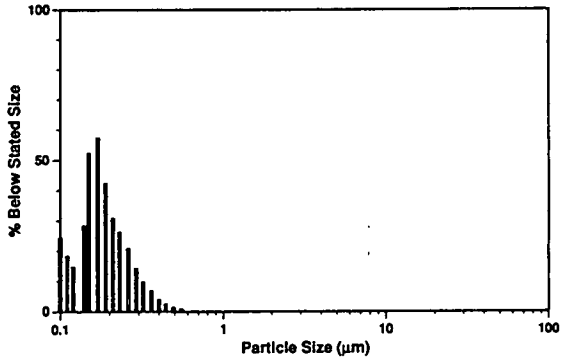

Figure 10. Droplet diameter distribution of MF59-100. MF59-100 was homogenized at 12,000 psi in the Microfluidizer® Model 110Y and size distribution was determined by laser light scattering with a Malvern Mastersizer X. Number average distribution is presented.

obtained by homogenization at pressures ranging from 6000 to 16,000 psi is shown in Fig. 9. The droplet diameters and moderate dependence of mean droplet diameter on operating pressure are consistent with data reported for the second generation microfluidized SAF emulsion (Lidgate *et al.*, 1992). Homogenization at 12,000 psi was adopted for continued development and emulsions were further characterized by light scattering with a Mastersizer X (Malvern Instruments, Southborough, MA) in order to determine contaminating levels of 1- to 10-μm droplets. Number-averaged data showed no detectable droplets in this range (Fig. 10).

In order to determine optimal process conditions for the preparation of adjuvant, 5-mL lots of MF59-0 were prepared by the previously described homogenization method using a number of process variations: (1) cooling to maintain product temperature at 25°C; (2) cooling to maintain product temperature at 40°C; (3) substitution of two serial passes for the recycle mode. The products were sterile filtered at the end of the runs through a 0.22-μm filter at a positive pressure of < 20 psi. The acceptability of the process was based on filterability of the product and yield of squalene as assessed by reverse-phase high-pressure chromatography (RP-HPLC) for the final squalene content. Results obtained from the series of runs are summarized in Table V. Adjuvants prepared at 25°C using the end-to-end process with two passes were somewhat difficult to filter, though no losses in squalene were observed. In contrast, emulsions made at 40°C using the recycling mode could be filtered at constant flow rates, and consistent lots of adjuvant emulsions were prepared and filtered with no change in composition. Therefore, a process employing 40°C runs at 12,000 psi using the recycling mode was adopted for the manufacture of 5-L lots of adjuvant.

Initial test vaccines were based on two vial formulations. Antigen was stored at –80°C and adjuvant at 2 to 8°C. Vials were mixed immediately before use. Therefore, a preliminary stability study was conducted with MF59-0 emulsion alone in either water for injection or 10 mM sodium citrate, pH 6.0, stored with or without nitrogen overlay for a period of 3 months at 4, 25, and 37°C. Adjuvant formulations stored at 2 to 8°C maintained pH and squalene content. Based on visual and microscopic appearance, morphology was unchanged during the study. On the other hand, emulsions prepared and stored in water at

Table V
Effects of Manufacturing Conditions on the Filterability and Composition of Adjuvant Emulsions

Sample	Volume	Run type	Unfiltered/ filtered	Bath/product temp.	Percent squalene	Percent of starting	Filterability
1	5 L	Recycle	Unfiltered	4/28°C	3.891 ± 0.010	91.7%	Difficult
			Filter 1		3.495 ± 0.048	81.4%	
			Filter 2		3.310 ± 0.027	77.1%	
2	5 L	Recycle	Unfiltered	4/28°C	4.454 ± 0.003	104%	Difficult
			Filtered		4.284 ± 0.051	99.8%	
3	5 L	Recycle	Unfiltered	25/38°C	4.530 ± 0.033	105%	Constant flow
			Filtered		4.350 ± 0.015	101%	
4	5 L	Recycle	Unfiltered	25/38°C	4.404 ± 0.015	103%	Constant flow
			Filtered		4.228 ± 0.045	98.5%	
5	5 L	Recycle	Unfiltered	25/38°C	4.259 ± 0.070	99.2%	Constant flow
			Filtered		4.228 ± 0.045	90.5%	
6	5 L	Recycle	Unfiltered	25/38°C	4.382 ± 0.051	102%	Constant flow
			Filtered		4.094 ± 0.005	95.4%	
7	5 L	End-to-end	Unfiltered	25/38°C	4.220 ± 0.039	98.3%	Constant flow
			Filtered		3.940 ± 0.021	91.8%	

25 or 37°C showed a drop in pH during storage and a significant squalene loss. When the adjuvant was buffered in citrate, however, there was no drop in pH or loss in squalene at the higher temperatures in the presence or absence of nitrogen. It is of interest to note that stabilization with the citrate buffer was superior to nitrogen overlay of the vial. The mechanism of the pH drop observed with the emulsions in water is currently under investigation.

Two formats were tested for use with vaccine formulations for advanced clinical trials: a single-vial liquid format containing MF59 and antigen in buffer and a dual-vial liquid with antigen and adjuvant stored separately. In either case an acceptable formulation was required to be stable at 2 to 8°C for at least 1 year. Two stability problems with these formulations needed to be addressed. Antigens had been stored at –80°C for Phase I trials and required development of buffers suitable for 4°C storage. In addition, no data existed on long-term compatibility of antigen(s) with MF59. A 90-day stability study was conducted with the MF59/HSV gB2, gD2 vaccine at 4, 25, and 37°C in order to evaluate the stability of the single-vial formulation in comparison with the corresponding two-vial formulation. The formulations tested are described in Table VI. Citrate buffering was previously shown to provide both pH stability and protection against loss of squalene. The addition of glycine inhibits formation of nonreducible gB2 oligomers presumably by competition with lysine and additional sodium chloride is required to avoid protein adsorption at low antigen inputs. A minimum set of stability-indicating assays was used. Emulsion stability was assessed by determination of visual appearance, pH, squalene content (RP-HPLC), and droplet diameter (photocorrelation spectroscopy). Antigen stability was assessed by determination of protein content (BCA), molecular weight (SDS

Table VI
Vaccine Compositions

Component	Concentration (mg/mL)
HSV single-vial vaccine composition	
Squalene	21.5
Tween 80	2.5
Span 85	2.4
Sodium chloride	4.7
Trisodium citrate dihydrate	2.68
Citric acid monohydrate	0.19
Glycine	10.0
gB2	0.060
gD2	0.060
WFI q.s. to	1.0 mL
HSV double-vial vaccine compositions—antigen	
gB2	0.120
gD2	0.120
Sodium chloride	9.4
Trisodium citrate dihydrate	2.68
Citric acid monohydrate	0.19
Glycine	20.0
WFI q.s. to	1.0 mL
HSV double-vial vaccine compositions—MF59	
Squalene	43.0
Tween 80	2.5
Span 85	2.4
Trisodium citrate dihydrate	2.68
Citric acid monohydrate	0.19
WFI q.s. to	1.0 mL

PAGE), and conformation (ELISA). Critical data from the comparative study are presented in Table VII. These data indicate that both formulations were unchanged over the 90-day period at either 4 or 25°C. Antigens showed only minimal signs of degradation by SDS PAGE after 3 months at 37°C. No loss of sterility was observed for any sample.

Immunogenicity studies conducted in guinea pigs showed that the 90-day formulations gave equivalent anti-gD2 and anti-gB2 ELISA titers as the day 0 samples, as well as indistinguishable virus neutralization responses (Fig. 11). Thus, the potencies of the vaccines were retained in both formulations. An additional stability study conducted to simulate transport conditions confirmed that these formulations would survive conditions of shipping. Based on these experimental data and outcome of an analysis of the key product criteria such as convenience in shipping, storage, and ease of clinical administration, the single-vial liquid formulation was chosen as the prototype formulation for future clinical trials.

Adjuvant emulsion formulations, with or without MTP-PE, have been generated in 5- to 10-L quantities for clinical trials. Also, a useful single-vial liquid formulation

Table VII

Summary Results from the 3-Month Stability Study with MF59/HSV gB2–gD2 Vaccine Formulations

Stability criteria	Single-vial liquid	Two-vial format (antigen & buffered MF59-0)
Droplet diameter	Remained the same for formulations at 4, 25, and 37°C.	Remained the same at all temperatures.
Visual/microscopic appearance	Remained homogeneous. Few large particles were seen under microscope at 3 months and at 37°C.	MF59-0 in citrate was homogeneous by visual appearance. Few large particles under microscopic exam at 37°C and 3 months.
Squalene content	No compositional change at 4 and 25°C.	No change in squalene at 4 and 25°C.
Protein (SDS-PAGE)	No evidence of proteolysis at 4 and 25°C.	No evidence of proteolysis at 4 and 25°C.
ELISA	ELISA showed no drop for gD2 and gB2 with respect to time zero measurements.	ELISA showed no detectable changes in either antigen with respect to time zero activity.
pH	Stable.	No changes in the buffered adjuvant or antigens.

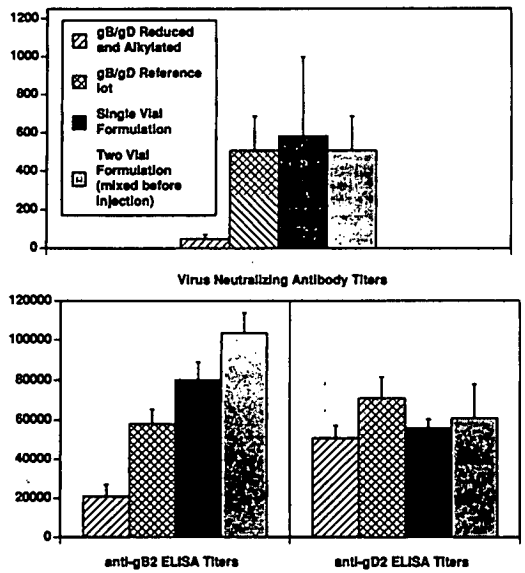

Figure 11. Virus neutralizing and ELISA antibody titers obtained in stability studies. Four groups of eight guinea pigs were immunized twice at a 2-week interval with MF59/gB,gD formulations which had been held 90 days at the time of the first injection. Reduced and alkylated gB2/gD2 were used with MF59 as a negative control for the virus neutralization assay.

containing the adjuvant and antigen(s) has been designed, and it provides physicochemical stability sufficient for performance of preclinical and clinical investigations. Further scaleup of the manufacturing process to produce 50- to 100-L quantities of the adjuvant and optimization of the different vaccine formulations are in progress.

5. CLINICAL RESULTS WITH THE MF59 ADJUVANT FORMULATION

The MF59 adjuvant formulation has been tested in over 3000 human volunteers in combination with recombinant HSV glycoproteins (gB and gD), recombinant HIV envelope proteins, and with commercially available influenza vaccines. These studies have been performed in both seronegative and seropositive volunteers with the HSV and HIV vaccines. Study populations have included normal adults (HSV, HIV, influenza) as well as elderly populations (influenza) and children and infants (HIV). The safety and immunogenicity of the MF59 adjuvant formulation have been clearly demonstrated in these clinical trials.

In HSV and HIV seronegative studies, recombinant antigens combined with the MF59 formulation generated high antibody responses as measured by ELISA or by virus neutralizing assays. In many cases the antibody titers achieved by vaccination with viral antigens combined with MF59 approached or even surpassed titers seen in seropositive, naturally infected individuals. In HSV trials, the antibody titers generated using MF59 were significantly higher than titers generated using alum. Very strong helper T-cell responses (lymphocyte proliferation) were also induced in HSV and HIV seronegative subjects with the MF59 formulation. In the seronegative populations, humoral and cellular responses to the vaccine were not enhanced when MTP-PE was included in the MF59 formulation. The MF59 formulation was also effective in stimulating immune responses with HSV, HIV, and influenza vaccines in seropositive subjects. Of interest, the addition of MTP-PE to the vaccine in HIV-seropositive individuals resulted in marked increases in HIV antigen-specific lymphocyte proliferation compared to results obtained in the absence of the muramyl peptide. This is the only circumstance where MTP-PE has been shown to have an immunological advantage over the emulsion-based formulation alone.

The clinical trials with MF59-0 have demonstrated that the adjuvant formulation is safe and well tolerated. The local reactions associated with the MF59 vaccine formulations include pain and tenderness at the injection site, and at a much lower frequency, erythema and induration which generally resolve within 24 to 48 h of immunization. Systemic reactions include a flu-like syndrome characterized by arthralgias, myalgias, headache, fever, and malaise. These symptoms also tend to resolve within 24 to 48 h of immunization. There does not appear to be a clear-cut relationship between local and systemic reactions associated with one immunization in terms of predicting the same side effect profile on subsequent immunizations. Other side effects that were noted very infrequently include transient elevation in liver function tests and rash. Safety has been clearly established with regard to hematologic parameters as well as renal function. The reaction profiles noted in HIV-1 seronegative subjects in a Phase II trial of gp120 MN with aluminum hydroxide versus gp120 SF2 with MF59 show identical low rates of reactions (mild local and systemic), further documenting the safety of the MF59 adjuvant. Ongoing Phase II prophylactic and Phase I/II therapeutic trials with HIV vaccine, Phase II trials with

influenza vaccine, and Phase III prophylactic and therapeutic trials with HSV vaccines will continue to document the safety of the MF59-0 adjuvant formulation.

Early trials with influenza vaccine, HIV vaccine, and HSV vaccine have shown that the addition of MTP-PE to the MF59 adjuvant formulation results in increased rates of local and systemic reactions over those seen in the absence of the muramyl peptide. In particular, a majority of individuals immunized with influenza vaccine or HIV env 2-3 developed moderate to severe local and systemic reactions which generally resolved within 24 to 48 h. There did not appear to be a dose–response relationship in terms of reactogenicity when graded amounts of MTP-PE were used in vaccine trials using the env 2-3 antigen. Of interest, subjects who had local and systemic reactions associated with the MF59/MTP-PE vaccine formulation had better tolerance to the vaccine in subsequent immunizations when the MTP-PE was removed from the formulation. No evidence of uveitis (by slit lamp examination) was seen in any subjects receiving vaccines containing MF59-0 or MF59/MTP-PE.

In summary, the MF59 formulation is a safe and highly immunogenic adjuvant when used in combination with a variety of recombinant and natural subunit antigens derived from HSV, HIV, and influenza viruses. Both humoral and cellular responses have been elicited with the MF59 formulations. The addition of the muramyl peptide, MTP-PE, provides no obvious immunological advantage in seronegative subjects but does increase the antigen-specific T-cell responses in HIV-seropositive individuals and does increase reactogenicity of the vaccine.

6. SUMMARY

MF59 is a safe, practical, and potent adjuvant for use with human vaccines. The formulation is easily manufactured, may be sterilized by filtration, and is both compatible and efficacious with all antigens tested to date. MF59 has been shown to be a potent stimulator of cellular and humoral responses to subunit antigens in both animal models and clinical studies. Toxicology studies in animal models and Phase I–III studies in humans have demonstrated the safety of MF59 with HSV, HIV, and influenza vaccines.

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